0017-R/M-P

Cytosine-5 methylation of ribosomal RNA in cell cycle control and migration

JUDITH LÓPEZ, SANDRA BLANCO

Centro de Investigación del Cáncer e Instituto de Biología Molecular y Celular del Cáncer(USAL-C Translational and Clinical Research Program

5-methylcytosine (m5C) is a widespread modification in DNA and RNA. However, while the functions of m5C in DNA have been extensively studied, its role in RNA is emerging to be elucidated (1)we focus on the occurrence of 5-methylcytosine (m5C deposition is found mainly in transfer RNA (tRNA) and ribosomal RNA (rRNA) and is mediated by DNMT2 and NSUN family members (1–4). Recently it has been shown that m5C on tRNAs regulates stem cell functions and stress responses in normal epidermis and skin cancer (5–7), and its inhibition specifically eliminates cancer initiating cells (5), suggesting that RNA-methylation may regulate essential cellular and physiological processes and its dysregulation may lead to critical pathological consequences such as cancer.

By analysing cancer expression databases we have found that the cytosine-5 methylase NSUN5 is overexpressed in advanced metastatic prostate cancer (PCa), one of the most frequent form of cancer Worldwide in men. *In vitro* analysis using *NSUN5*-silenced cell lines showed that *NSUN5* depletion leads to impaired proliferation and migration capacity. Flow cytometry analysis showed that *NSUN5* silencing results in decreased cell size and arrest in G2/M phase, suggesting an impairment in cell cycle progression through G2/M phase transition. We find that NSUN5 expression fluctuates along the cell cycle, further confirming its role in regulating this process. NSUN5 is a rRNA m5C methyltransferase that methylates position C3872, located at the interphase of the small and large ribosome subunits. Whether rRNA m5C methylation is regulated along the cell cycle and whether m5C deposition at rRNA regulates protein translation in G2/M and migration capacity needs to be determined.

1. R. Garcia-Vilchez et al. Biochim Biophys Acta Gene Regul Mech 1862, 240 (2019). 2. S. Hussain et al. Genome Biol 14, 215 (2013). 3. L. Van Haute et al. Nat Commun 7, 12039 (2016). 4. Y. Motorin et al. Nucleic Acids Res 38, 1415 (2010). 5. S. Blanco et al. Nature 534, 335 (2016). 6. J. V. Flores et al. Stem Cell Reports, (2016). 7. M. C. Popis et al. Curr Opin Oncol 28, 65 (2016).

0023-R/M-P

A possible key target for blocking glioblastoma progression: chaperone-mediated autophagy in pericytes.

MARIA LUISA MOLINA, MARIA BOTIA, ANA MARIA CUERVO, FERNANDO MACIAN, SALVADOR MARTINEZ, RUT VALDOR

University of Murcia/IMIB-Arrixaca Internal Medicine Department/ Brain regionalization and development gene unit

The lack of knowledge of the pathogenesis and the progression mechanisms of Glioblastoma (GB), the most aggressive brain tumor, contributes to none successful therapeutic strategies. Our team has recently demonstrated a crucial new role for chaperone-mediated autophagy (CMA) in pericytes (PC)-acquired immunosuppressive function during GB progression. GB-induced CMA in PC is necessary for proteostasis that promotes interaction with GB and, therefore, for an immunosuppressive function that facilitates tumor progression. Objective: to provide knowledge about the regulation and functional consequences of GB-induced CMA in PC. Methods: studies of RNA-seq and proteomics has been done in GB-conditioned pericytes with and without CMA compared to control pericytes after 72 hours of co-culture. Results: We have found several gene expression pathways differentially enriched in LAMP2A-KO PC and affected by GB-induced CMA in PC that correlate with our previous findings. Our data show that the phagosome formation, cell senescence, focal adhesion and the effector function to promote anti-tumor immune responses are the most affected pathways, reveling some transcription factors, as positive regulators of these processes that might be degraded by GB-induced CMA in pericytes, leading to facilitate GB progression. Conclusion: our results identify gene expression signaling pathways and possible new molecular markers that drive to the consequences of an aberrant upregulation of GB-induced CMA in PC and therefore, lead to “permissive” immune niche in the progression of GB.

Valdor R, García-Bernal D, Riquelme D, Martinez CM, Moraleda JM, Cuervo AM, Macian F, Martinez S. Glioblastoma ablates pericytes antitumor immune function through aberrant up-regulation of chaperone-mediated autophagy. Proc Natl Acad Sci U S A. 2019 Oct 8;116(41):20655-20665. doi: 10.1073/pnas.1903542116. Epub 2019 Sep 23

0033-R/M-P

MAPT is not just a protein of the Nervous System: Presence of Tau in the kidney

LAURA VALLÉS, FÉLIX HERNÁNDEZ, JESÚS ÁVILA

Centro de Biología Molecular Severo Ochoa Molecular Neuropathology

Tau is a cytoskeletal protein that is expressed mainly in neurons and is involved in several cellular processes such as microtubule stabilization, axonal maintenance and transport. Altered tau metabolism is related to different tauopathies being Alzheimer disease one of the most relevant, in which aberrant hyperphosphorylated and aggregated tau is found in the central nervous system. In this project, we have explored tau expression in peripheral tissues in Tau knock-out mice (B6.129S4 (Cg) -Mapttm1 (EGFP) Klt/J), which have an eGFP-coding sequence inserted into the first exon of the microtubule-associated protein tau gene. IVIS Lumina from PerkinElmer demonstrated eGFP expression mainly in the kidney. We then demonstrated by qPCR that the main tau isoform in the kidney is Tau4R. Thanks to the eGFP reporter we have been able to see that tau is found in the glomeruli of kidney cortex, and specifically in podocytes. This was further confirmed by immunohistochemistry. Tau-KO mice present a podocyte cytoskeleton more dynamic as they contain higher levels of detyrosinated tubulin than wild-type mice. In addition, transmission electron microscopy studies demonstrated glomerular damage. Our results demonstrate that tau has an important role in podocyte architecture under normal physiological conditions.

1- (Chang, Shao & Mucke, 2021) 2- (Gödel et al., 2015) 3- (Tucker, Meyer & Barde, 2001) 4- (Wang & Mandelkow, 2015) 5- (Xu et al., 2014)

0054-P

Unravelling the Implications of Two Pathogenic Mutations of the Apoptosis Inducing Factor in its NADH–oxidase Properties

NEREA NOVO, MILAGROS MEDINA, PATRICIA FERREIRA

Universidad de Zaragoza Bioquímica y Biología Molecular y Celular

The human apoptosis inducing factor (hAIF) is a moonlighting FAD–dependent enzyme that renders an essential role in the bioenergetics and redox metabolism of mitochondria in healthy cells, but which may also trigger caspase–independent cell death upon pro–apoptotic stimuli. hAIF dimerizes after reduction of its FAD cofactor by the NADH coenzyme, prompting the formation of a remarkably stable FADH–:NAD^+ charge transfer complex (CTC) (Ferreira et al., 2014). The monomer–dimer equilibrium that is hence established can be envisaged as a sensor of the mitochondrial redox state in terms of the NADH/NAD^+ levels, being further arbitrated by the allosteric binding of a second non–catalytic NADH molecule (Ferreira et al., 2014). Defects in hAIF give rise to major dysfunctions in oxidative phosphorylation, resulting in human pathogenic disorders coursing with severe neurodegeneration amongst other considerable symptoms. In the present work, we have performed the biophysical characterization of two mutations recently identified and related to disease (Heimer et al., 2018): Met340Thr and Thr141Ile, localized in the protein NADH–dependent and FAD–dependent domains respectively. In order to elucidate their participation on the reported pathological phenotypes, we have evaluated the impact of these mutations on NADH oxidase activity, CTC stability, overall protein stability, and interaction with key biological partners (DNA and the proteins CHCHD4, H2AX and CypA).

Ferreira, P. et al. (2014). Structural insights into the coenzyme mediated monomer-dimer transition of the pro-apoptotic apoptosis inducing factor. Biochemistry, 53(25), 4204–4215. https://doi.org/10.1021/bi500343r Heimer, G. et al. (2018). Mutations in AIFM1 cause an X-linked childhood cerebellar ataxia partially responsive to riboflavin. European Journal of Paediatric Neurology, 22(1), 93–101. https://doi.org/10.1016/j.ejpn.2017.09.004

0055-P

Study of the neuroinflammatory status of new cellular models of chronic IGF-1 deficiency

PAULA TIRADO-MELENDRO[1], ISABEL VARELA-NIETO[2], LOURDES RODRÍGUEZ-DE LA ROSA[3]

[1]Institute for Biomedical Research Alberto Sols CSIC-UAM, [2]Institute for Biomedical Research Alberto Sols (CSIC-UAM). CIBERER (ISCIII) Hospital La Paz Institute for Health Research (IdIPaz), [3]Biomedical Research Networking Center on Rare Diseases (CIBERER), ISCIII. IIBm (CSIC-UAM) Hospital La Paz Institute for Health Research (IdIPaz)

Human IGF-1 deficiency is a rare disease (OMIM608747) that causes sensorineural hearing loss (SNHL) and neurological disorders (Rodríguez-de la Rosa *et al.*, 2017). The *Igf1*-deficient mouse recapitulates this syndrome and shows impaired neuronal differentiation, along with early apoptosis of auditory neurons (Camarero *et al.*, 2001; Cediel et al., 2006). IGF-1 has pleiotropic actions, including decreasing neuroinflammation and promoting cellular senescence (Nishizawa *et al.*, 2016). To further study IGF-1 deficiency and understand the alterations linked to neuronal loss, a cellular model of the human disease was generated in the murine neuroblastoma cell line Neuro-2a using CRISPR/Cas9 technology. This model reproduces the partial deletion of exon 3 of the murine *Igf1* gene, a deletion that has been associated with human and mouse SNHL. For gene editing, the crRNA:tracrRNA:Cas9 complex was transfected as a ribonucleoprotein and the cell clones from a selected pool isolated using limiting dilution. *Igf1* gene editing was confirmed by Sanger and next-generation sequencing and two cell clones were selected to carry out the study, 4A10 and 2G3. Gene expression of IGF system components by RT-qPCR confirmed the absence of *Igf1* mRNA and revealed that *Igf1r* was downregulated in both clones. Comparative cell viability XTT assays using cisplatin, hydrogen peroxide and IGF-1 showed differences between wild type (WT) and edited clones. Neuro-2a WT cells showed the highest viability in response to FBS and IGF-1 treatment. Interestingly, both clones were more resistant to cisplatin, suggesting that the ablation of the